Study Title:

Request for Waivers of Inhalation Studies for Silver Dihydrogen Citrate Products

Data Requirements:
OCSPP 870.3465: 90-Day Inhalation Toxicity
OCSPP 875.1400: Applicator Inhalation Exposure – Indoor
OCSPP 875.1600: Applicator Exposure Monitoring Data Reporting

Study Completion Date:
September 26, 2020

Author:
Erik Janus
Steptoe & Johnson, LLP

Submitter:
ETI H2O, Inc.
771 Jamacha Road #512
El Cajon, CA 92019

Report ID:
ETI-2020.01

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this report on the basis of its falling within the scope of FIFRA Section 10(d)(1)(A), (B), or C). The submission of this report in compliance with FIFRA does not constitute a waiver of any right to confidentiality that may exist with respect to any other statute in the U.S., or in any other country.

Company: ETI H2O, Inc.

Company Agent/Submitter: 

Dolana Blount
VP, Product Development/Regulatory Affairs

Date: 09.26.2020

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This volume provides the rationale for waiver requests. The following exposure and risk assessment information is not subject to the principles of the U.S. Environmental Protection Agency's Good Laboratory Practice (GLP) Standards as set forth in 40 CFR Part 160.

Submitter: _____

Dolana Blount
VP, Product Development/Regulatory Affairs

Date: 09.26.2020

Request for Waiver of Guideline Numbers 870.3465, 875.1400 and 875.1600 for Silver Dihydrogen Citrate Products

Waivers are requested from the requirements to conduct 870.3465 (90-day inhalation toxicity), 875.1400 (inhalation exposure – indoor), and 875.1600 (applicator exposure monitoring data reporting) as originally requested by the July 6, 2012 Generic Data Call-In (GDCI-072500-1194) and reinstituted in September 2019. Per the letter from EPA, dated September 3, 2019, all the ETI H2O silver dihydrogen citrate products have been reclassified from Silver (elemental), PC Code 072501, to Silver ion, PC Code 072500.[1]

Introduction & Background

In the 2019 letter, EPA reinstituted its previous request that the repeated dose (90 day) inhalation toxicity testing (870.3465), the indoor applicator exposure data (875.1400), and the applicator exposure monitoring data reporting (875.1600) be submitted on the silver-containing antimicrobial products. Per the 2012 GDCI, the Agency has stated that it will accept a 28-day study to satisfy the requirement for 870.3465 (see the 2012 GDCI, Footnote 28) and only "required if there is the likelihood of significant repeated inhalation exposure to the pesticide as a gas, vapor, or aerosol" (see 2012 GDCI, Footnote 4). For 875.1400, these data are required for both occupational and residential sites if applied indoors (see 2012 GDCI, Footnotes 21,24); however, "[b]iological monitoring data may be submitted in addition to, or in lieu of, dermal and inhalation exposure data, provided the human pharmacokinetics of the pesticide and/or metabolite/analog compounds (i.e., whichever method is selected as an indicator of body burden or internal dose) allow for the back calculation to actual dose" (see 2012 GDCI, Footnote 26).

ETI H2O, a division of PURE Bioscience, produces four (4) products containing silver dihydrogen citrate.[2] The MUP concentrate (Product 1 below) contains 2400 ppm or 0.240% silver ion and 20.66% citric acid with the remainder consisting of deionized water, produced in an integrated system. Silver *per se* is not present in an isolated form; it exists only as an ion stabilized by citric acid. There is no particulate matter in these products, nor are there any nanoscale particles of silver.[3]

The four registered products are:

1. Axenohl (Reg. No. 72977-1) which is 2400 ppm or 0.240% silver ion and 20.66% citric acid. This is the manufacturing use product (MUP).
2. Axen30 (Reg. No. 72977-3) which is 30 ppm or 0.003% silver ion, 4.8% citric acid
3. Axen50 (Reg. No. 72977-4) which is 50 ppm or 0.005% silver ion, 5% citric acid.
4. SDC3A (Reg. No. 72977-5) which is 30 ppm or 0.003% silver ion, 4.8% citric acid.

---

[1] See letter from EPA to Steptoe & Johnson LLP, dated September 3, 2019. Subject: Amendment to the Generic Data Call-In Notices for Products Containing Silver Particles.

[2] See 74 FR 27745, July 10, 2009, where the Agency identifies this product as "Silver ions resulting from the use of electrolytically-generated silver ions stabilized in citric acid as silver dihydrogen citrate."

[3] The original DCI requirements from 2012 were placed on hold in 2015 pending an EPA review intended to determine whether existing registered products should be reclassified as "nanosilver." See letter from EPA from September 3, 2019.

These products are a MUP for making antimicrobial end-use products and ready-to-use sprays and solutions for indoor use. Application of the product is achieved via non-aerosol-generating-method (i.e. spray trigger, pump spray, etc.).

Most importantly, EPA waived the requirement for acute inhalation toxicity testing on Axenohl (Reg. No. 72977-1) and Axen products on June 21, 2001 (Data Package D274823). A waiver was also granted for acute inhalation toxicity testing on Axen 30 (Reg. No. 72977-3) with the following rationale provided by EPA (December 31, 2002 - Data Package D286175):

- Based on product formulation, the acute toxicity of Axen 30 is expected to be similar to what the citric acid component would be, at its concentration of 4.8%. This is far less than the concentration which the Citric Acid Reregistration Eligibility Document (RED) characterizes as mild with respect to systemic acute toxicity for any expected exposures from pesticide uses.
- Based on studies conducted on the applicant's Axenohl (EPA Reg. No. 72977-1, having similar components but much more concentrated than Axen 30)—which were accepted by Product Science Branch (PSB) in support of Toxicity Category IV for acute oral and acute dermal toxicity and skin irritation (February 3, 2001 PSB review, Data Package D270802)—the acute effects of Axen 30 are generally expected to be quite mild.
- The product is not expected to aerosolize upon use and is not expected to be volatile and thus does not produce a gas, vapor or aerosol.
- There is low potential for inhalation exposure: no aerosol application appears on the product label and no caution words are required on the label with regard to acute inhalation toxicity.

The antimicrobial action of silver or silver compounds is proportional to the bioactive silver ion released. Silver metal ionizes in the presence of water, body fluids or tissue exudates producing silver ions that can then exert their antimicrobial activity (Lansdown, 2006). Because silver metal ionizes in the presence of water or body fluids, it is expected that the toxicity of silver metal would be similar to that of silver ion in animal testing. Therefore in this document, studies on metallic silver and silver ions are summarized and used in the weight-of-the-evidence (WOE) rationale for waiving the requirement for repeated dose (90-day) inhalation and applicator exposure testing on ETI H2O's silver dihydrogen citrate (SDC) antimicrobial products.

Regulatory Basis For Requested Waivers

At 40 CFR 158.45, the Agency identifies that waivers for data requirements can be considered:

> a) The data requirements specified in this part as applicable to a category of products will not always be appropriate for every product in that category. Some products may have unusual physical, chemical, or biological properties or atypical use patterns which would make particular data requirements inappropriate, either because it would not be possible to generate the required data or because the data would not be useful in the Agency's evaluation of the risks or benefits of the product. The Agency will waive data requirements

it finds are inappropriate, but will ensure that sufficient data are available to make the determinations required by the applicable statutory standards.

The Agency's strategic objective for toxicity testing clearly identifies the Agency's objective of "Refining and reducing animal testing by maximizing information obtained from animal studies, and focusing on effects of concern" and moving from testing for 'completeness' to carefully focused animal testing where concerns exist, using ..."hazard based hypotheses about the plausible toxicological potential of a pesticide or group of pesticides based on their physical-chemical properties."[4] This is consistent with the National Academies of Sciences recommendations to EPA and is an essential component of the Agency's objectives.[5]

In the case of silver and silver salts, there is adequate information from other routes of exposure, across varied durations of exposure, and in both experimental animals and humans to conclude that silver and silver salts will not pose a toxicological concern from a subchronic or chronic inhalation exposure. There is no evidence of any adverse toxicological effects and no systemic toxicity from either oral or dermal exposure, nor is there any evidence from acute inhalation exposures. In fact, EPA has previously asserted that additional animal data is not relevant for regulation of silver, on the basis of protecting exposed human populations against argyria, a "cosmetic," non-toxicological endpoint associated solely with oral and inhalation exposures.[6]

Since 2012, EPA has not only refined its approach to accepting waivers and has not only issued multiple guidance documents and policy statements[7] but has also established an internal decision-making body that meets regularly to decide on submitted waivers using a published "weight of evidence" (WOE) process.[8] This internal decision-making body within the Office of Pesticide Programs (Hazard and Science Policy Council, or HASPOC) considers requests for waivers from repeat-dose studies (i.e. non-acute studies) and applies the WOE approach as defined by previous guidance. This approach includes specific consideration of physicochemical properties, use and exposure patterns, hazard characterization (i.e. toxicity profile), and implications for risk assessment.[9] When considering exposure and risk implications for non-dietary exposures, a margin of exposure (MOE) is derived and is compared to a regulatory level of concern (LOC) and, specifically for HASPOC waiver decisions, this "threshold for MOEs is typically 10x times the LOC."

This waiver follows the WOE format that the HASPOC prefers and comparison of estimated exposure levels to the HASPOC LOC (using an MOE approach) is presented in Section 4 below.

---

4 As identified at https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/strategic-vision-adopting-21st-century-science (formerly, http://www.epa.gov/pesticides/science/testingassessment.html)

5 Ibid.

6 See 74 FR 27447; June 10, 2009.

7 For example see the 2013 Guiding Principles for Data Requirements at https://www.epa.gov/pesticide-registration/guiding-principles-data-requirements and the 2013 Part 158 subchronic inhalation data requirements at https://www.epa.gov/sites/production/files/2014-02/documents/part158-tox-data-requirement.pdf.

8 See Craig, E. et al. (2019). Reducing the need for animal testing while increasing efficiency in a pesticide regulatory setting: Lessons from the EPA Office of Pesticide Programs' Hazard and Science Policy Council. Regul. Toxicol. Pharmacol. 108:104481. doi: 10.1016/j.yrtph.2019.104481. Epub 2019 Sep 20.

9 Ibid.

Weight-of-Evidence Rationale for Requested Waivers

1. Physico-chemical Properties of SDC Products

ETI H2O's SDC products contain no particulate silver, only silver ions stabilized in citric acid. For silver dihydrogen citrate, silver ions exist only in association with citric acid (not in isolation) and are present only at 1.16% of the citric acid level. Because silver is not systemically toxic, any testing with the highest concentration product (i.e., Reg. No. 72977-1, concentrate containing 0.24% silver ion and 20.66% citrate) would evaluate only the citric acid component[10], which the Agency has already determined not to be a concern from subchronic inhalation exposure (Citric Acid RED, U.S. EPA, 1992).

As discussed in the exemption from requirement for a tolerance for SDC products, EPA considers the ETI H2O products distinct from metallic silver and silver salts and described thusly: "silver ions resulting from the use of electrolytically-generated silver ions stabilized in citric acid as silver dihydrogen citrate (does not include metallic silver)."[11] Furthermore, silver ion "does not volatilize [and] any post-application inhalation exposures to vapors are expected to be negligible."[12]

The EPA previously waived the acute inhalation testing requirement for SDC products, due to the nature of the product formulation, anticipated mild acute effects, lack of sufficient volatility, inability of the product to aerosolize and no aerosol application on the label.

2. Use and Exposure Patterns of SDC Products

The registered SDC MUP (Reg. No. 72977-1) is used solely to produce ready-to-use sprays and solutions for indoor use that contain 0.003 to 0.005% silver ion stabilized in 4.8% to 5% citric acid. Application of the product is achieved via non-aerosol-generating-method (i.e. spray trigger, pump spray, etc.). The label directions for the SDC end-use products specify that the product is applied until the surface is thoroughly wet. The surface may then be wiped with a clean towel after 30 seconds-10 minutes (depending on the target organism).

No aerosols are generated during the application of the product, as application is by non-aerosol generating methods (spray trigger, pump, etc.). There is very low inhalation exposure potential ("negligible" post-application exposure to vapor) from label-directed use of SDC products.

---

[10] The ratio of silver ion to citrate in this product is 1:86, therefore whatever toxicity is exhibited in a repeated dose inhalation study is likely to be related to citrate and not to silver ion, itself. The ratio in the marketed, ready to use products is even greater ranging from 1:1000 to 1:4000. Because of the large ratio of silver ion to citrate, ETI H2O does not understand why testing on silver should be required for this product. Resulting toxicity, if any, would be due to citrate and not to silver, which has already been well characterized.

[11] See 74 FR 27448, June 10, 2009.

[12] Ibid, p. 27451.

Any future inhalation studies in which particulate effects are possible (which would be due to the species of silver used as the test material) would overestimate the potential effects of a non-particulate form of silver and would not be representative of potential SDC exposures.
There are no national dietary studies in the US that measure silver in populations; however, the national public health authorities in both Canada and France have published results of human biomonitoring surveys that do measure silver. The Canadian Health Measures Survey measures the blood levels of many thousands of citizens each cycle and reported in 2013 that the median and 95th upper percentile silver blood concentrations were 0.066 and 0.27 µg/L, respectively.[13] The French agency ANSES measured the level of silver in general population diets in 2008 and reported that mean exposure in adults was 1.29 – 2.65 µg/kg/day and 1.6 – 3.47 µg/kg/day for children.[14]

3. Hazard Characterization (Toxicity Profile of Silver Ion)

The overall toxicity of silver ion is very low and well-characterized from multiple lines of evidence as described below and this has been recognized in multiple technical and regulatory documents for silver in general as well as for SDC products. Specifically for the silver ion in SDC products, it has been stated that "humans and laboratory animals do not handle elevated doses of silver in the same manner," and thus, "additional conventional laboratory animal toxicity studies would not provide a better understanding of the effects of silver in humans." (74 FR 72447, at 27449).

Silver poses minimal toxicity upon inhalation. This is supported by several lines of evidence, including: (1) human epidemiological studies as described below, which do not show any effects other than those related to argyria (a cosmetic endpoint of no toxicological concern[15]); and (2) animal studies with non-nanoscale silver which are sparse yet include examples of silver being used as a therapeutic agent for respiratory conditions.[16] Once absorbed via inhalation, silver is not extensively metabolized and reacts with certain specific chemical groups found in proteins (like glutathione and sulfhydryls) to form "extremely stable silver selenide and silver sulfide complexes that are effectively non-bioavailable" and these are mainly eliminated via feces by the liver and kidney.[17]

---

[13] Health Canada 2013. Second Report on Human Biomonitoring of Environmental Chemicals in Canada. Available: www.healthcanada.gc.ca/biomonitoring.

[14] [ANSES], French agency for food, environmental and occupational health and safety. 2011. Second French Total Diet Study (TDS2). Inorganic contaminants, minerals, persistent organic pollutants, mycotoxins and phytoestrogens. Available from: https://www.anses.fr/en/system/files/PASER2006sa0361Ra1EN.pdf.

[15] Per 74 FR 27447, "there is no animal conditions that would mimic the dermatologic form argyria found in humans following exposure to silver by various routes."

[16] The vast majority of available silver inhalation studies use nanoparticulate silver and these data are largely irrelevant to the toxicity of silver ion; however, many of these studies often use silver ion (from silver chloride, silver nitrate, etc.) as a comparator.

[17] See Morrow M., EPA OPP Antimicrobials Division (2009). Ionic Silver: Toxicity and Weight of The Evidence. Dated May 11, 2009. Also, see Walker. M. and D. Parsons (2012). The biological fate of silver ions following the use of silver-containing wound care products – a review. *Int. Wound J*. doi: 10.1111/j.1742-481X.2012.01115.x.

A study on the distribution of inhaled metallic silver in dogs was conducted (Phalen and Morrow, 1973). Six female Beagle dogs (9.6 to 13.2 kg) were administered a single acute inhalation exposure of metallic radiolabeled silver aerosol by tracheal intubation, and clearance and distribution of the silver were determined. Aerosols were generated using an exploded wire technique from silver wires that contained about 30 µCi of 110mAg/mg of silver. The dogs inhaled the material for 7-15 minutes. The aerosols were agglomerates with a mass median diameter between 0.42 and 0.54 µ. The mass median diameter of unaggregated particles was 0.04 µ. Most of the radioactivity was deposited in the liver, followed by lung, brain, skin and muscle. Clearance data were analyzed into exponential components or fractions of the total cleared. The lung had biological clearance half-lives of 1.7, 8.4 and 40 days accounting for 59, 39 and 2% of the total deposited silver, respectively.

The available human and rat physiologically-based pharmacokinetic models[18] for silver ion confirms that soluble silver species are transformed to silver sulfide and stored in tissue, with an oral absorption fraction of ~ 5 % and estimated inhalation absorption fraction of ~20% for the rat. It should be noted that due to lack of inhalation absorption data in the literature, the PBPK model was used to estimate the 20% absorption fraction in the rat based on a particle inhalation model[19] that relied in part on *in vivo* studies using silver nanoparticles. The Bachler PBPK model allows for the prediction of blood and urinary levels of silver ion following oral, inhalation, intravenous or dermal exposures; however, since very little retained silver is excreted via urine[20] then this leaves the blood concentrations as the more useful biomarker.

Aylward et al. (2016)[21] used the Bachler PBPK model to derive a Biomonitoring Equivalent (BE)[22] for silver, which is a steady-state biomarker concentration consistent with a regulatory point of departure (POD). The BE model is "run by chronic steady-state input of the dose at the human equivalent POD and the resulting predicted steady-state blood concentration arising is identified as the BE_{POD}." This value is divided by various uncertainty factors as called for by specific PODs, such as a BE_{RFD}. Specifically for silver, Aylward et al. (2016) simulated blood concentrations from intravenous exposure using the Bachler PBPK model to derive the BE_{POD}, mirroring the derivation of the EPA RfD, which was done using human biomonitoring data.[23] The human

---

18 As reported by Bachler, G., N. von Goetz and K. Hungerbuhler (2013). A physiologically based pharmacokinetic model for ionic silver and silver nanoparticles. *Int. J. Nanomed*. 8: 3365-3382.

19 ICRP Human respiratory tract model for radiological protection. A report of a Task Group of the International Commission on Radiological Protection. *Ann ICRP*. 1994;24(1–3):1–482.

20 Jiménez-Lamana, J. et al. (2014). An insight into silver nanoparticles bioavailability in rats. *Metallomics*. 6(12):2242-9.

21 Aylward, L.L., G. Bachler, N. von Goetz, D. Poddalgoda, S.M. Hays and . Nong. (2016) Biomonitoring Equivalents for Interpretation of Silver Biomonitoring Data in a Risk Assessment Context. *Int. J. Hyg. Environ. Health*. 219(6): 521-526.

22 Originally introduced by Hays SM, Becker RA, Leung HW, Aylward LL, and Pyatt DW. (2007). Biomonitoring equivalents: A screening approach for interpreting biomonitoring results from a public health risk perspective. *Regulatory Toxicology and Pharmacology*, 47(1), 96–109. Health Canada has published (and uses) an identical approach to directly compare "BEs with results from Canadian population biomonitoring studies [with] the potential to inform decision makers if current exposures are approaching or exceeding tolerable levels for the general population." (See https://www.canada.ca/en/services/health/publications/science-research-data/biomonitoring-equivalents-screening-tool-population-level-data.html)

23 This is described in Morrow (2009) at Footnote 12.

equivalent POD was 0.014 mg/kg/day, from which the BE_{POD} of 1.32 µg/L was derived; this BE_{POD} was derived by a total UF of 3 to yield the BE_{RFD} of 0.4 µg/L. Therefore, blood concentrations of less than 0.4 µg/L (or 0.00004 mg/dL, 0.4 ppb) would be considered "safe" as consistent with the EPA RfD.

The results of worker exposure studies on silver are summarized below followed by the results of studies conducted in experimental animals. In worker exposure studies, there have been respiratory complaints however, they do not occur at levels that cause argyria and/or argyriosis. In addition, it has been claimed that the respiratory effects observed were seen primarily in smokers or ex-smokers which would suggest that silver exposure was not involved or only to a minimal degree in those effects. Finally, the workers were exposed to multiple substances, complicating interpretation of the various study results. The results of the experimental animal studies support the findings in the worker exposure studies, indicating the low toxicity of inhaled silver.

Human Epidemiological Studies

Several occupational exposure studies are available on various forms of silver. Rosenman, et al. (1979)[24] conducted a study on workers from a small plant in upstate New York that were exposed to silver nitrate and silver oxide. Thirty individuals were included in the study. All were male except for one female office employee. A little over half of them (16) had worked at the plant for 5 or more years. Six individuals had argyria and 20 had argyrosis (deposition of silver in the eye). Four months before the study, six production workers wore personal air samplers on two separate 8-hour workdays. Time weighted average ranged from 0.039 mg/m3 to 0.378 mg/m3 by atomic absorption spectrometry. The highest values were seen in two workers involved in silver oxide production. The exposure values were all above the OSHA standard of 0.01 mg/m3. Most of the workers complained of eye and both upper (nose and throat) and lower (cough, wheezing and chest tightness) respiratory irritation. Nose bleeding was also noted while working in the plant, as were skin burns in those exposed to silver nitrate. Chest radiograms and results of clinical examination of respiratory function were predominantly normal, with no demonstrated relationships between abnormalities and duration of employment. Average blood levels of silver ranged from 1.8 µg/100 cc blood (range 1.9 to 5.7) in those with < 1 year of employment to 36 µg/100 cc blood in those employed from 1.1 to 9.9 years. Those employed for 10 or more years had blood levels of 2.5 µg/100 cc blood.

Abdominal pain was also reported by these workers. The pain was described as "burning in quality and relieved by antacids" and was reported in 10 out of 30 workers examined. This symptom correlated significantly with blood silver levels, indicating that those workers exposed to higher levels of airborne silver nitrate and/or oxide were more likely to suffer gastrointestinal pain. The symptomology reported by the workers in Rosenman et al. (1979) is consistent with effects seen following inhalation of certain nitrogen compounds as well as the caustic nature of silver nitrate. For example, large oral doses of silver nitrate can cause severe gastrointestinal irritation and acute exposure to nitrogen dioxide has been reported to cause coughing, shortness of breath, chest pain/tightness, headache, dizziness, nausea and vomiting.[25]

---

[24] Rosenman, K.D., A. Moss, and S. Kon. Argyria: Clinical Implications of Exposure to Silver Nitrate and Silver Oxide, *J. Occ. Med.* 21: 430-435, 1979.

[25] The effects of silver nitrate exposure are discussed in Casarett & Doull's Toxicology (5th Edition, 1999).

In another study conducted by Rosenman et al. (1987)[26], 27 workers at a company that manufactures silver and other precious metal powder were examined. Metals exposed to included silver, cadmium, gold, platinum and palladium. The powders produced were silver nitrate, silver oxide, silver chloride and silver cadmium oxide powders. The mean time the individuals worked at the plant was 8.1 years (range <2 to over 10 years. Fifteen of the workers complained of mucosal irritation such as itchy, red or watery eyes, sneezing, runny or stuffy nose or sore throat. Eight of the workers complained of nose bleeds. Urine silver and cadmium levels were measured as were blood silver levels. Mean urinary silver was 11.3 µg/L (upper limit for unexposed groups <1.9 µg/L), mean blood silver was 10 µg/100 ml (upper limit for unexposed groups <0.27 µg/ 100 ml) and mean urinary cadmium levels were 11.8 µg/L (upper limit for unexposed groups <10 µg/L). Respiratory complaints had no statistical relation with the results of biological monitoring.

The respiratory irritation reported in the Rosenman studies was considered by Drake and Hazelwood (2005)[27] to be inconclusive because silver oxide and nitrate, by nature, are irritants. As such, the irritancy effects could not be attributed to silver per se.

Workers (37) exposed to silver at an Eastman Kodak Company were monitored (DiVincenzo et al., 1985)[28]. An additional 35 workers at this plant who were not exposed to silver served as the controls. The average age of both study groups was about 45 years and the mean duration of employment for both groups was about 20 years. Atmospheric and personal samples were collected and analyzed for silver. The 8-hour time weighted average exposure to silver over a 2-month monitoring period (62 samples ranged from 1-100 µg/m3. Blood, hair, feces and urine were collected from the silver exposed and control groups and analyzed for silver content. Mean blood and urine levels of silver were not detectable in the controls. Mean fecal and hair samples contained 1.5 and 0.57 µg/g, respectively. The silver workers had levels of 0.011 µg/ml in blood, undetectable levels in urine, 15 µg/g in the feces and 130 µg/g in hair. Because silver is eliminated predominantly in the feces, fecal measurements were used as an index of exposure and as a means of calculating body burdens. At the TLV of 0.1 mg/m3, fecal excretion of about 1 mg of silver is expected per day. In this study, silver workers excreted an average of 0.3 g per day in feces, which would equate to a time weighted average workplace exposure of about 0.03 mg/m3. The study authors concluded that generalized argyria is unlikely to occur in workers exposed to silver at the above exposure levels. The hair levels were determined to partially be due to silver bound to hair from the atmosphere and not from absorption of silver. The study did not report any adverse findings in the silver exposed workers and no occurrences of argyria. It can be implied from the study authors conclusions that argyria was not seen in these workers.

[26] Rosenman, K.D., Seixas, N., and I. Jacobs. Potential Nephrotoxic Effects of Exposure to Silver. *Brit. J. Ind. Med.* 44:267-272, 1987.

[27] Drake, P. and K. Hazelwood. Exposure-Related Health Effects of Silver and Silver Compounds: A Review. *Ann. Occup. Hyg.* 49: 575-585, 2005.

[28] DiVincenzo, G.D., Giordana, C.J., and L.S. Schriever. Biologic Monitoring on Workers Exposed to Silver. *Int. Arch. Occup. Environ. Health.* 56: 20-215, 1985.

Pifer et al. (1989)[29] conducted a study in the same Eastman Kodak plant. A cross-sectional investigation was conducted on 27 employees occupationally exposed to primarily insoluble silver compounds and 27 matched controls employed at the same plant. The average age of the silver workers was 46.2 years. The mean duration of employment was 19.3 years (range 6 to 39 years). The controls' average age was 45.6 years and their average length of employment was 20.4 years. The estimated annual exposure rate was 10 to 200 µg silver/m3 (8-hour basis, unadjusted for the use of respiratory or facial protection). These levels were based on area samples and person dosimetry. Facial photography revealed no evidence of argyria when comparing the silver exposed workers to the non-exposed workers. Electron microscopy of the granular and basement membrane structures and of the staining pattern of collagen provided no evidence of silver in any of the biopsies. There was indication of silver deposits in the eyes, but there was no vision impairment. Respiratory systems were reported in two silver workers and two of the controls. Three had a history of smoking and the cough experience by the fourth individual cleared up with antibiotic treatment. No significant differences between the two groups were seen upon pulmonary function testing and no findings were seen in the silver workers upon chest radiography. The mean concentration of silver was 0.01 µg/ml among the silver workers who had measurable blood silver levels. Silver was not detected in the blood of any of the controls. Detectable silver in the urine was observed in only one silver exposed worker. Both controls and silver exposed workers had detectable levels of silver in feces. The study authors concluded that there were no chronic effects of silver in these silver exposed workers. They also noted that in the Rosenman et al. (1979) study, respiratory obstructive changes were essentially limited to smokers and ex-smokers even though their exposure levels were higher than those in this study.

The following was extracted directly from Drake and Hazelwood (2005)[30]:

An unpublished report obtained from Johnson Matthey (Linnett and Bradford, 1996) discusses a study of 41 workers from the United Kingdom, who were involved in the recovery and recycling of silver. None of the workers showed signs of argyria or argyrosis even though past exposure to metallic silver exceeded 0.1 mg/m3. Their length of employment ranged from 3 months to 29 years. Median exposure in 1976–1977 was 0.25 mg/m3, and the geometric mean from 1987 to 1996 was 0.52 mg/m3. The researchers concluded that metallic and soluble forms of silver should be distinguished when setting exposure limits and that 0.1 mg/m3 is a safe exposure level for metallic silver to prevent argyria.

Inhalation Toxicity of Silver in Experimental Animals

Inhalation toxicity studies in animals are not available on silver salts; however, a 2-hour inhalation study in rabbits is available on colloidal silver and a 5-day inhalation study on silver (I) coupled to a methylated caffeine carrier (SCC1, a sustained silver release product; Cannon, et al. 2009[31])

[29] Pifer, J.W., Friedlander, B.R., Kintz, R.T. and D.K. Stockdale. Absence of Toxic Effects in Silver Reclamation Workers. *Scand. J. Work Environ. Health*. 15: 210-221, 1989.
[30] Ibid., 27.
[31] Cannon, C.L., Hogue, L.A., Vajravelu, R.K., Capps, G.H., Ibricevic, A., Hindi, K.M., Kascatan-Nebioglu, A., Walter, M.J., Brody, S.L. and W.J. Youngs. In Vitro and Murine Efficacy and Toxicity Studies of Nebulized SCC1, a Methylated Caffeine-Silver (I) Complex, for Treatment of Pulmonary Infections. *Antimicrob. Agents Chemother.* 53: 3285-3293, 2009.

is available. Nebulized SCC1 is being developed for the treatment of pulmonary infections in humans. A similar product, SCC10 is also being developed for treatment of cystic fibrosis and pulmonary infections in humans (Hindi, et al 2009[32]). In this study, male mice (C57BL/6J; 8-10 week old) were exposed to nebulized water or SCC1 (5 mg/dose) for a period of five minutes twice a day for 5 days. On a mole basis the dose was 29% silver. The particle sizes of SCC1 to which the mice were exposed ranged from 1-5 μm. The total exposure time over the 5-day period was 1.5 hours. Mice were weighed daily. Their behavior in the chamber was monitored by photography from an aerial view and every 15 seconds beginning 5 seconds after the onset of the aerosol. The effect of the methylated caffeine carrier without silver was also monitored to determine whether any effects observed were due to the carrier alone. At the end of the five days, the mice were sacrificed and the lungs were removed for histological examination.

The mice exposed to aerosolized water approached the nebulizer and then moved to the opposite side of the chamber. Once the chamber equilibrated, the mice dispersed throughout the chamber. The SCC1 exposed mice moved away from the mist and congregated at the opposite end of the chamber and dispersed throughout the chamber once chamber equilibration took place. Mice treated with the carrier alone (previously exposed to the water aerosol), went to the opposite end of the chamber away from the nebulizer, but not as quickly as the SCC1-exposed mice. The animals exhibited no weight loss or changes in grooming behavior. Although the mice displayed avoidance behavior, the lungs from the treated mice appeared histologically normal with no evidence of increased immune cell infiltration.

In 2007, the FDA cleared the use of silver-coated endotracheal breathing tubes for the prevention of pneumonia in patients using ventilators.[33] In a laboratory study, it was found that silver was rapidly released from silver-coated endotracheal tubes. The release was described by a mono-exponential function with a time-constant tau of about 60 minutes and a saturation concentration of 200 + 80 μg/ or 0.2 mg/L.[34] Within the first 10 minutes, concentrations released were 50 μg/L (0.05 mg/L). Based on the results of this laboratory study, silver concentrations in the lungs of humans in which these tubes are used could be as high as 0.2 mg/L at saturation. These levels were considered to be safe for use in patients by the FDA.

In an older rabbit study, 8 healthy adults of both sexes (3 months old; 2 to 3 kg) were used (Konradova, 1968a[35] and 1968b[36]). Only three of the rabbits were exposed by inhalation for two hours to colloidal silver. Five rabbits served as the control group. A saturated solution of silver

---

[32] Hindi, K.M., Ditto, A.J., Panzner, M.J., et al. The antimicrobial Efficacy of Sustained Release Silver-Carbene Complex-loaded L-tyrosine polyphosphate nanoparticles: Characterization, In vitro and In Vivo Studies. *Biomaterials* 30: 3771-3379, 2009.

[33] See http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/2007/ucm109023.htm.

[34] Hartman, M. Guttmann, J., Muller, B. et al. (1999) Reduction of the bacterial load by the silver-coated endotracheal tube (SCET), a laboratory investigation. *Techno. Health Care* 7:359-70.

[35] Konradova, V. The Ultrastructure of the Tracheal Epithelium in Rabbits Following the Inhalation of Aerosols of Colloidal Solutions of Heavy Metals. I. Changes in the Ultrastructure of the Tracheal Epithelium after 2-Hour Inhalation of Colloidal Solutions of Iron and silver. *Folia Morphol.* 16: 258-264, 1968(a).

[36] Konradova, V. The Ultrastructure of the Tracheal Epithelium in Rabbits Following the Inhalation of Aerosols of Colloidal Solutions of Heavy Metals. II. Signs of Cell Alteration in the Epithelium after 8 Hour Inhalation of Colloidal Solutions of Iron and Silver. *Folia Morphol.* 16: 265-271, 1968(b).

containing 10% argentum colloidale (colloidal silver) was used. Aerosols were prepared using a clinical glass inhaler devised by Synek. The effect of the aerosol on the ultrastructure of the tracheal epithelium was determined. According to the report, "[a]fter the inhalation, deep recesses and crypts filled with closely arranged functionless cilia were formed on the surface of the epithelium". Irritation to the goblet cells was noted and signs of apocrine mechanism of secretion of the goblet cells were found. Silver passed through the epithelium and was found in the vacuoles of the macrophages which appeared in large numbers under the basement membrane of the epithelium in the lamina propria mucosa. It was concluded that the test material, when it accumulates in the crypts, interferes with the self-cleaning mechanism of the tracheal epithelium, which may be related to the deposition in tissues and cells of silver, a well-known effect of silver in animal studies. This study was included in this document for completeness; however, due to the age of the study and absence of information on the size of the particles to which the rabbits were exposed and the concentration and exact composition of the test material, the relevance and usefulness of this study is questionable.

Arai et al. (2015)[37] exposed a group of three (3) male ICR mice (5 weeks old) and a separate group of eight (8) similar mice to a silver nitrate solution (at 10.3 µg silver per mouse) via intratracheal instillation. After 4 hours, 7% of this initial dose was recovered in the liver, and after 24 hours, liver weight was significantly decreased relative to controls. No silver was detected in the urine of mice treated with silver nitrate and the lung concentrations were measured at 4.08 ± 1.8 and 2.53± 1.35 µg silver per gram of tissue at 4 and 24 hours, respectively. The authors concluded that silver ion rapidly translocated from the lung to other tissues and systemic circulation after instillation.

Wen et al. (2016)[38] exposed a group (n=22) of newborn Sprague Dawley rats to silver ions daily to 1 mg/kg (2 mg/ml silver nitrate at 0.5 µl/g daily) for 4 weeks via intranasal instillation and a separate group (n=29) daily to 0.1 mg/kg (0.2 mg/ml silver nitrate at 0.5 µl/g daily) for 12 weeks with a 4-week recovery period. The authors found that significant retention of silver in the brain as a result of the intranasal delivery method (unlike inhalation which tends to lead to distribution to the lung, liver, and kidney preferentially); however, a low-effect and no-effect level on body weight decrease can be identified from this study. At the highest dose (1 mg/kg/day), a body weight decrease in both sexes was noted (LOAEL) yet no effect was found in either sexes at 0.1 mg/kg/day, which is the NOAEL from this study. It should be noted that the exposure period for the lower dose was 12 weeks, several weeks longer than a 90-day subchronic study.

4. Implications for Risk Assessment of SDC Products

Any additional animal data collected on silver is likely to be uninformative in support of risk assessment using argyria as the health endpoint of concern, per past EPA reviews and Federal Register announcements. The nature of the SDC product to be tested is such that any toxicity observed would be due to the large amount of citric acid/citrate in the formulation and not the

---

[37] Arai, Y., T. Miyayama, and S. Hirano (2015). Difference in the toxicity mechanism between ion and nanoparticle forms of silver in the mouse lung and in macrophages. *Toxicology* 328: 84-92.

[38] Wen, R., X. Yang, L. Hu, C. Sun, Q. Zhou and G. Jiang (2016) Brain-targeted distribution and high retention of silver by chronic intranasal instillation of silver nanoparticles and ions in Sprague-Dawley rats. *J. Appl. Toxicol.* 36: 445-453.

silver ion per se. The inhalation toxicity of citric acid is well documented and solutions of roughly 6% citric acid are used as a positive-control, "cough challenge" agent. Any future inhalation studies using the SDC MUP (with a citric acid content of roughly 20%) would clearly not be informative as to the toxicity of inhaled silver ion.
From past RASSB risk assessments of an SDC product containing 50 ppm (0.0005%) silver, AD has established:

1) An inhalation interim point of departure of 0.0003 mg/kg/day was established from the OSHA 8-hr TWA (based on argyria) and applying a safety factor of 3 for an incomplete toxicity database.
2) Residential and occupational inhalation exposures resulting from wiping and trigger pump application methods (all assuming an application rate of 4.17 E-04 pounds AI/gallon) ranged from 2.01 E-06 to 5.21 E-5 mg/kg/day (reported as absorbed daily dose).
3) Exposure factors from industry and PHED ranged from 1.3 to 2.4 mg/pounds AI (trigger pump application) to 67 mg/pounds AI (which was measured from finger pump application of a solution and then wiping it up).

By comparing the past risk assessment results with the HASPOC MOE process for making waver decisions, the most relevant maximum exposure barely exceeds what would be the presumed HASPOC LOC (10x less than the inhalation POD). Applying information on pulmonary absorption from the available silver ion PBPK model shows an anticipated, conservatively-derived exposure that is below the LOC and supports the decision to waive the subchronic inhalation and applicator exposure studies.

	POD (ppm)	HASPOC LOC (ppm)	Exposure Max (ppm)	HASPOC MOE
Inhaled Silver Ion (50 ppm)	0.0003	0.00003	0.0000521	1.7
Adjusted for 20% Pulmonary absorption	0.0003	0.00003	0.0000104	0.35

Another point of comparison to the HASPOC LOC could be the BE_{RFD} for silver, which was calculated by Aylward et al. (2016) to be 0.4 parts per billion (ppb). Maximum inhalation exposure from the RASSB risk assessment was 0.0521 ppb, which is lower than either the calculated BE_{RFD} and slightly higher than a presumed HASPOC LOC derived from this of 0.04 ppb.

Conclusions

The following lines of evidence justify a waiver from 870.3465, 875.1400, and 875.1600:

1) SDC products contain no particulate silver, only silver ions stabilized in citric acid (which is not a safety concern to EPA). Silver ion displays very low volatilization into the atmosphere.
2) The application methods for SDC products do not generate aerosols when used as labeled thus any inhalation exposures would be very low. No aerosol application appears on the

product label and no caution words are required on the label with regard to acute inhalation toxicity.

3) The overall toxicity of silver, once absorbed, is low and the only human-relevant effect of concern to date (argyria) is of no toxicological significance, other than cosmetic. Multiple lines of evidence support the conclusion that silver is poorly absorbed and any residual silver is complexed (such as silver sulfide) and not bioavailable.
4) The EPA previously waived the acute inhalation testing requirement for SDC products, due to the nature of the product formulation, anticipated mild acute effects, lack of sufficient volatility, inability of the product to aerosolize and no aerosol application on the label. The subchronic study requirement should be waived for all the same reasons.
5) Per EPA, "additional conventional laboratory animal toxicity studies would not provide a better understanding" and will be uninformative to risk assessment. Variables such as selection of test material (silver chloride, silver nitrate, etc.) and differential toxicokinetics/toxicodynamics between laboratory animals and man (animals process silver differently than humans) would obfuscate the results of any future animal-based studies. Furthermore, there are *in silico* approaches available to help generate any necessary points of departure for risk assessment.[39]
6) Waiver of the inhalation study (870.3465) precludes the need for any additional applicator or post-application exposure studies (875.1400, etc.) as inhalation toxicity from SDC products would be considered inconsequential and of no informative value to the risk assessment and/or registration decision-making process.
7) Past RASSB exposure estimates are very slightly above the HASPOC LOC and drop to below this level when taking into account information on pulmonary absorption from the silver ion PBPK model.

Taken together, the above lines of evidence indicate that no information relevant to the regulation of ETI H2O's silver dihydrogen citrate (SDC) products would be obtained by conducting a 90-day inhalation study in the rat. There is ample evidence to conclude that further testing with silver dihydrogen citrate would not provide any additional information relative to the evaluation of risks but, in fact, confirms that systemic toxicity from a subchronic exposure is highly unlikely.

Therefore, ETI H2O respectfully requests a waiver for this data requirement (870.3465) in addition to applicator exposure (875.1400) and the applicator exposure monitoring data reporting (875.1600) requirement for ***silver ions stabilized in citric acid as silver dihydrogen citrate***.

[39] Such as the PBPK model reported by Balcher et al. and referenced in Footnote 13.